Table S2
Determination of the concentration of active MBP-antiABA-scFv.

Response interval	Flow rate (µl·min <sup>-1</sup> )	$L_{\rm m} \times 10^{-5}$ (m·s <sup>-1</sup> )	$\frac{d\mathbf{R}/d\mathbf{t}_{\text{reg}}}{(\mathrm{RU}\cdot\mathrm{s}^{-1})}$	$\chi^2_{\rm reg}$	$\frac{dR/dt}{(RU \cdot s^{-1})}$	MTL
I: 100-200 RU	2	0.32	7.53	0.10	7.48	0.80
	5	0.43	9.53	0.04	9.63	0.75
$[A_{bulk}] = 32.098 \text{ nM}$	10	0.54	11.5	0.04	11.44	0.70
$L_r = 1.307 \times 10^{-5} \text{ m} \cdot \text{s}^{-1}$	25	0.73	14.02	0.01	14.03	0.64
$\chi^2_{\text{fit}} = 0.0048$	50	0.90	16.08	0.00	16.08	0.58
70 IR	100	1.1	17.3	0.01	17.28	0.54
II: 200-300 RU	2	0.32	7.23	0.03	7.13	0.80
	5	0.43	9.23	0.03	9.27	0.75
$[A_{bulk}] = 30.759 \text{ nM}$	10	0.54	11.1	0.04	11.11	0.73
$L_r = 1.290 \times 10^{-5} \text{ m} \cdot \text{s}^{-1}$	25	0.73	13.75	0.04	13.78	0.64
$\chi^2_{\text{fit}} = 0.0065$	50	0.73	15.75	0.04	15.76	0.58
λ fit-0.0003	100	1.1	17.35	0.09	17.25	0.55
III. 200 400 DII	2	0.32	7.08	0.02	7.03	0.78
III: 300-400 RU	5					
[A ] 20.701M		0.43	9.02	0.04	9.06	0.72
$[A_{bulk}]=30.791 \text{ nM}$ $L_r=1.1 \times 10^{-5} \text{ m} \cdot \text{s}^{-1}$	10	0.54	10.8	0.03	10.77	0.67
$\chi^2_{\text{fit}} = 0.002$	25 50	0.73 0.90	13.21	0.09	13.21 15.14	0.60
χ fit—0.002	100	1.1	15.08 16.31	0.06 0.08	16.27	0.54 0.51
IV. 400 500 DII	2	0.22	6.0	0.04	6 97	0.76
IV: 400-500 RU	2 5	0.32	6.9	0.04	6.87	0.76
[A 1 20 940]M		0.43	8.74	0.04	8.79	0.70
$[A_{bulk}]=30.849 \text{ nM}$ $L_r=1.00 \times 10^{-5} \text{ m} \cdot \text{s}^{-1}$	10	0.54	10.39	0.01	10.39	0.65
	25 50	0.73	12.7	0.07	12.65	0.58
$\chi^2_{\text{fit}}$ =0.002	50 100	0.90 1.1	14.37 15.41	0.13 0.21	14.41 15.43	0.52 0.49
V: 500-600 RU	2	0.32	6.97	0.07	6.77	0.73
	5	0.43	8.43	0.04	8.57	0.65
$[A_{\text{bulk}}]=30.692 \text{ nM}$	10	0.54	9.98	0.07	10.03	0.60
$L_{\rm r} = 0.846 \times 10^{-5} \mathrm{m \cdot s^{-1}}$	25	0.73	12	0.04	12.04	0.53
$\chi^2_{\rm fit} = 0.0145$	50	0.90	13.56	0.06	13.56	0.46
	100	1.1	14.5	0.07	14.43	0.41
VI: 600-700 RU	2	0.32	6.58	0.09	6.51	0.71
	5	0.43	8.11	0.11	8.19	0.66
$[A_{bulk}]=30.622 \text{ nM}$	10	0.54	9.52	0.07	9.54	0.61
$L_r = 0.7815 \times 10^{-5} \text{ m} \cdot \text{s}^{-1}$	25	0.73	11.41	0.04	11.38	0.54
$\chi^{2}_{\text{fit}}=0.0037$	50	0.90	12.81	0.15	12.76	0.48
•• ===	100	1.1	13.49	0.11	13.54	0.45

The slopes of sensorgram curves (dR/dt<sub>reg</sub>) at different flow rates were determined by linear regression in each chosen response interval (I-VI). The  $\chi^2_{reg}$  values correspond to the fit of the linear regression. The mass transport coefficient (L<sub>m</sub>) was calculated according to equation 1. The concentration of MBP-antiABA-scFv ([A<sub>bulk</sub>]) and the coefficient of reaction flux (L<sub>r</sub>) were determined by globally fitting the data obtained at different flow rates to equation 2, using dR/dt<sub>reg</sub> as slopes for each response interval. Ideal slopes (dR/dt<sub>fit</sub>) were obtained by introducing the calculated L<sub>m</sub>, L<sub>r</sub> and

A<sub>bulk</sub> values into equation 2. The measured concentration of *active* MBP-antiABA-scFv, calculated as the average of [A<sub>bulk</sub>] from each response interval, is  $30.97 \pm 0.559$  nM, which corresponds to 21.8% activity of the total purified protein in the sample (determined by measuring the optical density at 280 nm). The diffusion coefficient for MBP-antiABA-scFv was calculated to be  $5\times10^{-11}$  m<sup>2</sup>·s<sup>-1</sup>. The concentrations of MBP-antiABA-scFv determined by measuring the UV absorbance at 280 nm was  $142\text{nM}/10.6\mu\text{g·ml}^{-1}$ .